

WO 00/73781

13

PCT/AU00/00689

CLAIMS:

1. An oligonucleotide molecule for the detection of *Giardia lamblia* (*G. lamblia*), the oligonucleotide molecule hybridises under medium to high stringency conditions to unique 18S rDNA/rRNA sequences of *G. lamblia*.
2. The oligonucleotide molecule according to claim 1 which hybridises to unique 18S rDNA/rRNA sequences of *G. lamblia* under conditions of high stringency.
3. The oligonucleotide molecule according to claim 1 or 2 selected from the group consisting of oligonucleotides having the following nucleotide sequences:
- GCG TCC CGG GTG AGC GGG (SEQ ID NO: 1);
GCC CGC GGG CGC CCG CCC (SEQ ID NO: 2);
TGG GCC CGC CTC GCT CGC (SEQ ID NO: 3);
CGG CGG GGG GCC AAC TAC (SEQ ID NO: 4);
CGG GGT CCA ACG GGC CTG (SEQ ID NO: 5);
CGG GGC TGC CGC GGC GCG (SEQ ID NO: 6); and
oligonucleotides comprising a part of the sequences above having at least ten bases which hybridise to unique rDNA/rRNA sequences of *G. lamblia*.
4. The oligonucleotide molecule according to claim 3 comprising the nucleotide sequence:
- CGG CGG GGG GCC AAC TAC (SEQ ID NO: 4).
5. The oligonucleotide molecule according to claim 3 comprising the nucleotide sequence:
- CGG GGC TGC CGC GGC GCG (SEQ ID NO: 6).
6. The oligonucleotide molecule according to any one of claims 1 to 5 being detectably labelled.
7. The oligonucleotide molecule according to claim 6 wherein the label is selected from the group consisting of fluorochrome, radioisotope, and chemical.
8. The oligonucleotide molecule according to claim 7 wherein the label is a fluorochrome.
9. The oligonucleotide molecule according to claim 7 wherein the fluorochrome is selected from the group consisting of fluorescein isothiocyanate (FITC, green), cyanine dyes Cy2, Cy3, Cy3.5, Cy5, Cy5.5 (ranging from green to far red), and Texas Red.

10010211 042602

WO 00/78781

PCT/AU00/00689

14

10. A method for the detection of the presence of viable cells of *G. lamblia* in a sample, the method comprising the steps of:

- (a) adding to the sample a probe consisting of a detectably labelled oligonucleotide molecule which hybridises under medium to high stringency conditions to unique 18S rDNA/rRNA sequences of *G. lamblia*;
- (b) allowing hybridisation of the probe to the 18S rDNA/rRNA of any *G. lamblia* cells present in the sample; and
- (c) detecting hybridisation of the probe.

11. The method according to claim 10 wherein the probe is selected from the group consisting of oligonucleotides having one or more of the following nucleotide sequences:

GCG TCC CGG GTG AGC GGG (SEQ ID NO: 1)
GCC CGC GGG CGC CCG CCC (SEQ ID NO: 2)
TGG GCC CGC CTC GCT CGC (SEQ ID NO: 3)
CGG CGG GGG GCC AAC TAC (SEQ ID NO: 4)
GCG GGT CCA ACG GGC CTG (SEQ ID NO: 5)
CGG GGC TGC CGC GGC GCG (SEQ ID NO: 6)

and oligonucleotides comprising a part of the sequences above having at least ten bases which hybridise to unique rDNA/rRNA sequences of *G. lamblia*.

12. The method according to claim 11 wherein the oligonucleotide molecule comprises the nucleotide sequence:

CGG CGG GGG GCC AAC TAC (SEQ ID NO: 4).

13. The method according to claim 11 wherein the oligonucleotide molecule comprises the nucleotide sequence:

CGG GGC TGC CGC GGC GCG (SEQ ID NO: 6).

14. The method according to any one of claims 10 to 13 is used in combination with fluorescence *in situ* hybridisation (FISH) in which the oligonucleotide probe is labelled with fluorochrome and after hybridisation, the resulting fluorescent-labelled cell is detected by epifluorescence microscopy or flow cytometry.

15. The method according to any one of claims 10 to 14 wherein several different oligonucleotide probes are used and are distinguished by the use of different labels on each probe.

16. The method according to claim 15 wherein the oligonucleotide probes are labelled with different fluorochromes and detected by flow cytometry.

WO 00/78781

PCT/AU00/00689

15

17. The method according to any one of claims 10 to 16 wherein the sample is an environmental sample.

18. An oligonucleotide molecule which hybridizes to *G. lamblia* 18S rDNA/tRNA sequences under medium to high stringency conditions, wherein the oligonucleotide molecule hybridizes to at least one of target regions of *G. lamblia* rDNA having the following nucleotide sequences:

CCC GCT CAC CCG GGA CGC (SEQ ID NO: 7);

GGG CGG GCG CCC GCG GGC (SEQ ID NO: 8);

GCG AGC GAG GCG GGC CCA (SEQ ID NO: 9);

GTA GTT GGC CCC CCG CCG (SEQ ID NO: 10);

CAG GCC CGT TGG ACC CGC (SEQ ID NO: 11);

CGC GCC GCG GCA GCC CCG (SEQ ID NO: 12).

10018211.042602